BioChemistry

THE POTENTIAL ROLE OF THE PI3K/AKT PATHWAY IN ABNORMAL CELL GROWTH

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One of the characteristics of tumor cells is that they do not exhibit normal growth control. This can be studied in the laboratory by growing cells in serum-free medium. Many investigators have shown that normal human fibroblasts require serum for growth while human fibrosarcoma cell lines grow equally well in serum-containing or serum-free medium. Previous work in our laboratory, using a series of inhibitors to signal transduction proteins, has suggested that phosphatidylinositol 3-kinase (PI3K) may play a role in this abnormal growth. The experiments described below were designed to test the hypothesis that the PI3K/Akt pathway is involved in the abnormal growth of the fibrosarcoma cells in culture.

We attempted to test our hypothesis using two approaches. First, we studied fibrosarcoma cell strains that had been transfected with a plasmid coding for a dominant-negative form of PI3K. Our initial experiments were performed to determine if any of the cell strains we had isolated expressed the dominant-negative protein. The goal was to identify strains that expressed the dominant-negative protein, and then determine the rate of growth of these cells in serum-free medium. Our working hypothesis would predict that cells expressing the dominant-negative protein, which specifically inhibits the endogenous protein, would not grow as well in serum-free medium as cells transfected with the control plasmid. Unfortunately, results of Western blot analysis showed that none of our selected clones expressed the dominant-negative protein even though they expressed the geneticin resistant gene of the plasmid. We hypothesize that in growing the cells to establish stable transfectants, we selected for cells that expressed the geneticin resistant gene but not the dominant negative PI3K.

Our second approach was to measure the activity of Akt, a kinase downstream of PI3K in the pathway. This PI3K/Akt pathway has been shown to be involved in cell growth by a number of investigators. Our hypothesis would predict that the activity of Akt would likely be higher in the fibrosarcoma cell lines when compared to the normal fibroblasts. A commercial kinase kit from Cell Signaling Technology was used and the results clearly showed that the Akt activity in the two fibrosarcoma cell lines tested was significantly higher than that observed in the two normal human fibroblast cell lines that were analyzed. This data supports our hypothesis that the PI3K/Akt pathway is involved in the abnormal growth of these tumor cell lines. (Supported in part by the National Science Foundation Research Experiences for Undergraduates Grant #9820454.)